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Preliminary Investigations into the Interactions of Herbicides with Aqueous Humic Substances*

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Abstract: The binding of the herbicides to aqueous humic substances was investigated using the herbicide mecoprop and a reference humic substance 'Hohlohsee'. Initial results from 2D fluorescence spectrometry, infra-red spectroscopy and Curie-point pyrolysis gas chromatography/infra-red spectroscopy tentatively characterise the nature of the interaction.

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1 INTRODUCTION

Herbicides applied to agricultural land inevitably come into contact, and interact, with humic substances. A large proportion of dissolved organic carbon is present in the environment as macromolecular humic substances (HS). These humic substances contain phenol, carboxy, ether, acid and aromatic groups and are known to have exceptional complexing powers. A complete understanding of the binding and transport mechanisms of herbicides in the environment is essential when predicting their fate.

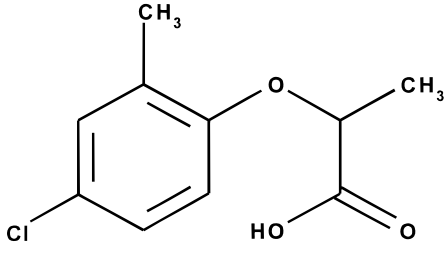
The results presented here are the product of an overlap between two special research areas 'Pesticides' and 'Characterisation of Humic Substances' within the Institute of Spectrochemistry in Dortmund.

In this study, investigations have been carried out into the interactions between mecoprop, a phenoxycarboxylic acid, and aqueous humic substances (DFG reference HS Hohlohsee, Germany, prepared by Abbt-Braun and Frimmel, Universität Karlsruhe and Burba and Aster, ISAS Dortmund).

Mecoprop (Fig. 1) was selected as a representative of an important class of herbicides which are difficult to determine in the free carboxylic form. The halogenated phenoxycarboxylic acids and their derivatives find widespread use as weedkillers in their salt and ester forms and are often applied in combination with other herbicides.

2 EXPERIMENTAL METHODS

The immediate interaction between the reference humic substance and the herbicide was followed in aqueous



mecoprop
(*R,S*)-2-(4-chloro-*o*-tolyloxy)propionic acid

Fig. 1. Chemical structure of mecoprop.

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solution using 2D fluorescence quenching spectroscopy. The identification of possible 'bound residues' was carried out using combined pyrolysis-gas chromatography/infra-red spectroscopy. Conventional potassium bromide salt pellet transmission spectra of the reference humic substance and the humic substance containing the bound herbicide residue were also measured.

2.1 Sample preparation

The reference humic substance (HS; DFG Hohlohsee) was dissolved (0·1 mg ml^{-1}) in deionised water (Seradest Vario/Seralpur Delta UV Fa. Seral, Ransbach-Baumbach, Germany). The acidity of the solution was adjusted to pH 3·0, 5·3, 7·0 and 9·0 using hydrochloric acid (0·1 M) and aqueous sodium hydroxide (0·1 M). All solutions were degassed with argon, stirring for 24 h. The pH values were checked again after this period and re-adjusted to the required value where necessary. The herbicide-treated sample was prepared in the same way as the reference sample except that mecoprop (Dr Ehrenstorfer, Augsburg, Germany) was also dissolved in the HS solution (~ 10 μg ml^{-1}).

A solid sample with 'bound' herbicide residue was obtained by ultra-filtration of the treated HS solution using 1000-Dalton filters (OMEGACELL Fa.Filtron, 50 Bearfoot Rd, Northborough, Mass.). The herbicide in solution passed through the filter whereas only HS particles of molecular mass >1000 Da remained behind on the filter. This was then washed several times with deionised water. Any herbicide recovered from the dried HS sample would have been bound to the HS at the time of filtering.

2.2 Fluorescence spectroscopy

The 2D fluorescence spectra were measured using a SPEX Fluoromax spectrometer (Instruments SA, München, Germany). This instrument consists not only of a scanning monochromator for the emitted fluorescence radiation but also a scanning monochromator for the excitation radiation. This makes locating the fluorophores in a complex sample such as a humic substance significantly easier. Complex changes to the fluorescence spectra caused by quenching by the herbicide where not only loss of emitted intensity but also shifts in the emission maxima with regard to both excitation and emission wavelength take place can now be followed. For this set of experiments the excitation wavelength was scanned between 200 and 600 nm. Emission collection was between 350 and 900 nm, although no additional information was observed above 700 nm. Both excitation and emission slits were set to 5 nm. A step size of 2 nm was selected with an integration time of 0.2 s per data point. Fluorescence signals were measured by a red-sensitive photomultiplier tube (Hamamatsu R928P) operating in photon-counting mode.

Spectra were corrected for wavelength-dependent changes in excitation intensity by dividing the emission signal by a reference signal obtained from a silicon photodiode placed after the excitation monochromator.

The sample solutions were placed in a 3-mm 90° quartz cell to obtain the reference and quenched 2D fluorescence spectra.

2.3 Infra-red spectroscopy

2.3.1 Curie-point pyrolysis gas chromatography/IR

Initial experiments were carried out into the determination of herbicide bound residues using Curie-point pyrolysis-GC/IR. The use of pyrolysis for the determination of the structural composition of humic substance is fraught with dangers so that special care must be taken when planning such experiments and when interpreting the results from such work.[1,2] The reference humic substance (100 ng) was placed in a quartz tube (Fisons, Wiesbaden, Germany) of the type typically found in use for sample delivery in mass spectrometry rather than directly onto the pyrolysis wire (Fig. 2). This novel method avoids reaction of the sample with the surface of the wire, resulting in much milder pyrolysis conditions. The sample was then rapidly heated in a helium shield gas atmosphere in the Curie-point pyrolysis attachment (Horizon Instruments Ltd, Heathfield, East Sussex, UK: oven temperature 200°C, pyrolysis time 5 s) to the gas chromatograph (Perkin Elmer 8500, Perkin–Elmer GmbH, Überlingen, Germany: Column: PTE5, Length: 30 m, ID: 0·25 mm, F.D.: 0·25 μm Supelco, Homburg, Germany: Column temperature programme: 70°C//7°C min^{-1}//200°C). The output gas flow of the capillary column was fed into a light-pipe attachment on a Perkin–Elmer 2000 FTIR spectrometer (Perkin–Elmer, Überlingen, Germany). Spectra were obtained with a resolution of 8 cm^{-1} from a fast, liquid-nitrogen-cooled, mercury cadmium telluride detector (MCT). The scan rate was 3 scans s^{-1} and one scan per data point.

As the aim of the experiment was to try to identify the species of bound residue, mild pyrolysis conditions were selected (358°C). Chromatography peak detection was carried out both on the FID trace of the gas chromatograph and on the Gramm-Schmidt chromatogram re-constructed from the infrared spectra recorded. Peak

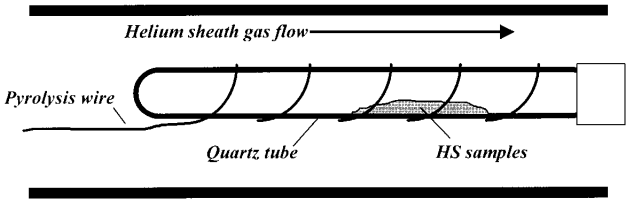

Fig. 2. Pyrolysis quartz tube and wire for mild pyrolysis conditions.

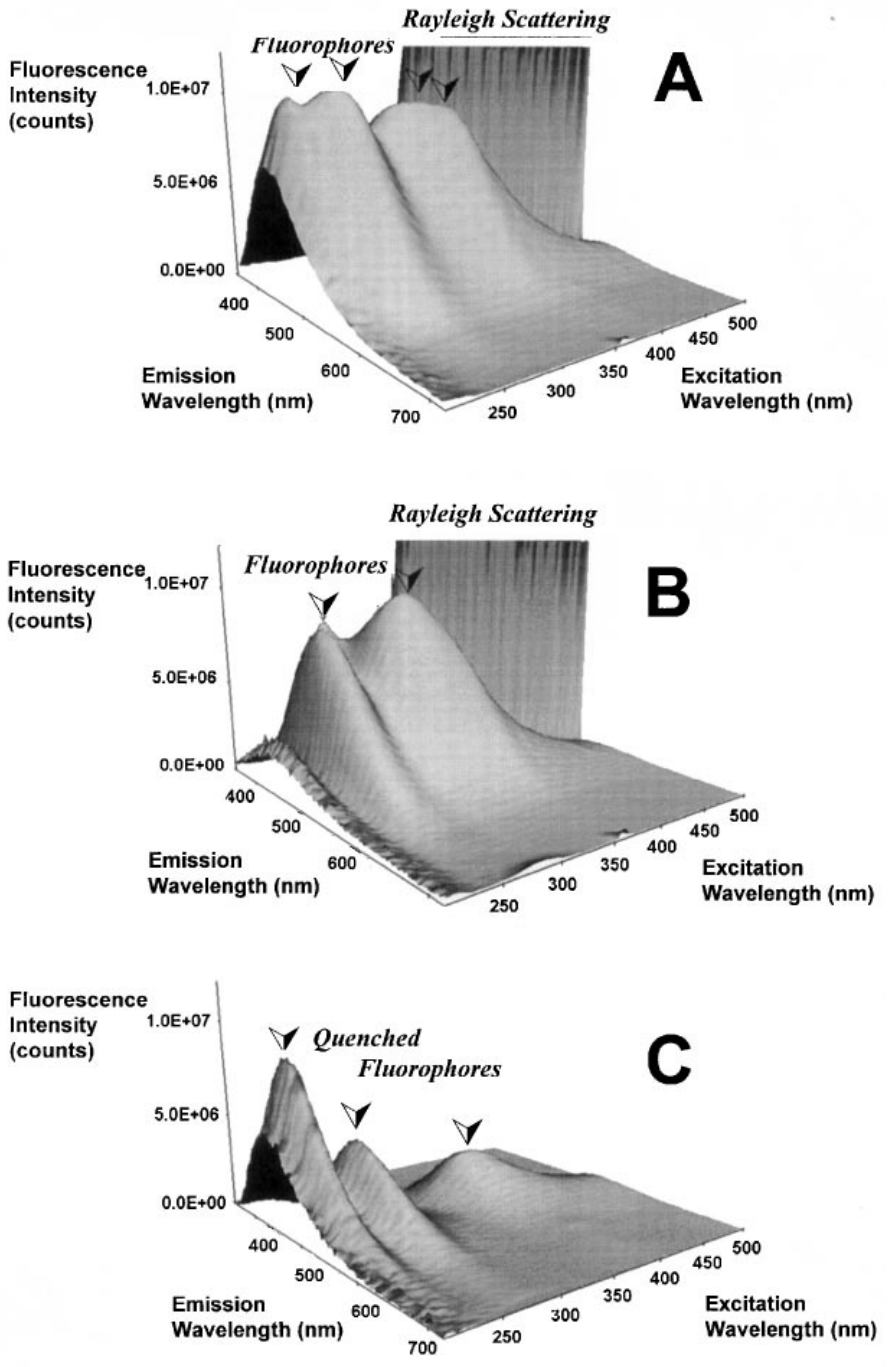

Fig. 3. 2D Fluorescence spectra of: A. reference HS Hohlohsee; B. as A, but with $\sim 10\ \mu g\ ml^{-1}$ mecoprop added; C. difference spectra showing the quenched fluorophores.

identification was made by searching a database of reference GC/IR spectra measured in-house.

2.3.2 Infra-red transmission spectroscopy

Following the conclusive identification of the residual mecoprop in the pyrolysis-GC/IR data, an attempt was made to locate the bound residue *in situ* on the humic substance by conventional infra-red transmission spectroscopy. The humic substance (1·5 mg) and potassium bromide (200 mg) were ground together before being pressed (10 tonnes pressure) for 10 min to form the salt pellet. Transmission spectra were obtained again on a Perkin–Elmer 2000 FTIR spectrometer, this time using a DTGS detector resolution of $4\ cm^{-1}$ 30 scans referenced against a potassium bromide pellet containing no additives.

3 RESULTS

3.1 2D Fluorescence spectroscopy

The 2D fluorescence spectra shown in Fig. 3 clearly show the difference between the fluorescence from a pure HS solution (A) and the fluorescence from an HS

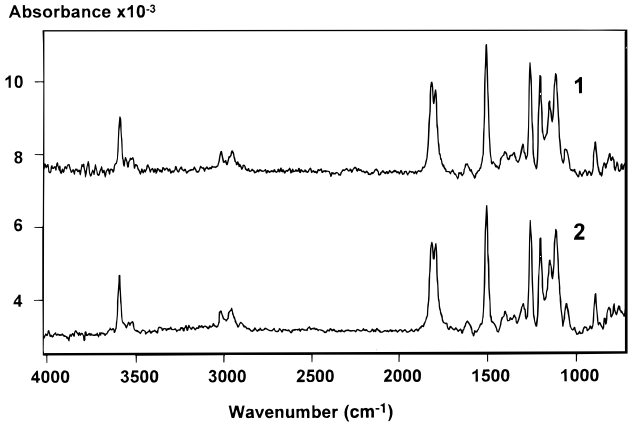

Fig. 4. Pyrolysis-GC/IR spectra of: 1. mecoprop identified as the pyrolysis product of an aqueous HS Hohlohsee sample treated with mecoprop and removed from solution by ultra-filtration; 2. reference mecoprop.

quenched by the addition of the herbicide mecoprop (B).

The difference spectrum shown as Fig. 3C was derived by subtracting the spectrum obtained with added herbicide from the reference HS spectrum. A strong preferential quenching of one of the fluoropores can be seen around 430 nm emission, 225 nm excitation in the reference humic substance Hohlohsee.

The data shown in Fig. 3 for pH 9·0 are typical for the other pHs studied except that increased herbicide absorption was observed with increasing pH.

3.2 Infra-red spectroscopy

3.2.1 Curie-point pyrolysis gas chromatography/IR

Initial experiments using Curie-point pyrolysis-GC/IR have yielded pure herbicide detection limits down to amounts of 250 ng absolute and have been able to detect the herbicide originally bound to the HS in aqueous solution (Fig. 4).[3] The mild pyrolysis conditions allow the 'bound' residue to detach from the humic substance without destruction of the herbicide molecular structure.

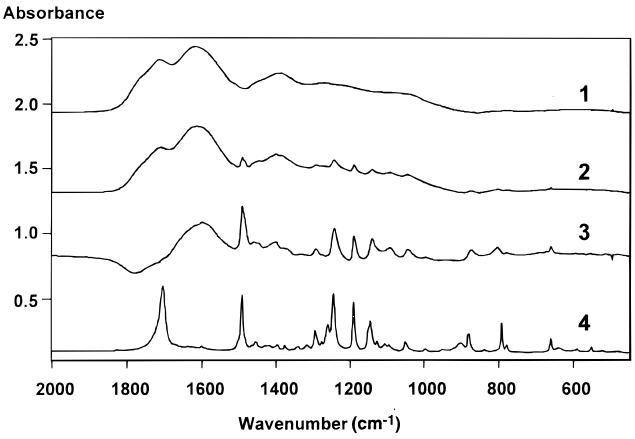

Fig. 5. Infrared potassium bromide pellet transmission spectra of: 1. pure HS Hohlohsee 1·5 mg/200 mg KBr; 2. as 1, but following treatment with mecoprop; 3. difference between 1 and 2 showing the bound mecoprop residue; 4. reference mecoprop spectrum 1 mg in 200 mg KBr.

3.2.2 Infra-red transmission spectroscopy

Conventional infra-red potassium bromide disc spectra of the reference humic substance are shown in Fig. 5 together with spectra of the HS heavily treated with mecoprop and the difference between the two. As the mecoprop-treated HS sample was separated from solution by ultra-filtration, the mecoprop identified was still bound to the HS. The infra-red spectra showed a loss of the acid carbonyl vibration which would be at 1710 cm^{-1}. This could be indicative of the mode of herbicide binding to the HS.[4]

4 CONCLUSIONS

A specific interaction between a reference humic substance and the herbicide mecoprop has been demonstrated. The 2D fluorescence quenching data show that the mecoprop is bound at specific site types, as only certain fluorophores are quenched. The results of the pH studies agree with early single wavelength studies on paraquat/HS interactions.[5] To clarify the nature of the herbicide/HS interaction the results of further studies currently underway into the identification of the source of the various HS fluorophores must be awaited.

The bound mecoprop residue has been detected using pyrolysis-GC/IR as well as conventional infra-red spectroscopy. This leaves open the question as to what degree a 'bound' herbicide residue should be regarded as being lost to an eco-system. The initial results, clearly identifying the herbicide separating from the HS, have recently been confirmed by GC/mass spectrometry (Nolte, J., pers. comm.). As the herbicide was observed desorbing from the HS in the original acid form it would suggest that the binding mechanism is not completely the result of herbicide degradation reaction with the HS as has been suggested in the literature.[6]

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REFERENCES

1. Saiz-Jimenez, C., Reactivity of the aliphatic humic moiety in analytical pyrolysis. *Organic Geochemistry*, **23** (1995) 955–61.

2. Hatcher, P. G. & Minard, R. D., Comment on the origin of benzenecarboxylic acids in pyrolysis methylation studies. *Organic Geochemistry*, **23** (1995) 991–4.
3. Malissa, Jr., H. & Winsauer, K., Erfassung von chlorierten Phenolen in Herbicid-Säuren mit der Capillar-GC/FTIR-Spektroskopie. *Fresenius Z. Anal. Chem.*, **318** (1984) 103–10.
4. Senesi, N., Free radicals in electron donor-receptor reactions between a soil humic acid and photosynthesis inhibitor herbicides. *Z. Pflanzenernaehr. Bodenk.*, **144** (1981) 580–6.
5. Olson, B. M., The use of fluorescence spectroscopy to study herbicide-humic acid interactions: Preliminary observations. *Can. J. Soil Sci.*, **70** (1990) 515–18.
6. Sensei, N., The role of humic substances in the behaviour of pesticides in soil: Interactions involving free radical species. In *Methodological Aspects of the Study of Pesticide Behaviour in Soil*. INRA Versailles, 16–17 June 1988.